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Evaluation of the protective effect of *Rhei Radix et Rhizoma* against α -naphthylisothiocyanate induced liver injury based on metabolic profile of bile acids

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Abstract

Ethnopharmacological relevance: To evaluate the hepatoprotective effect of the root and rhizome of Rhubarb (*Rhei Radix et Rhizoma*) against α -naphthylisothiocyanate (ANIT)-induced liver injury using metabolic profile of bile acids (BAs) along with biochemical parameters and histological alterations.

Materials and methods: Ultra-performance liquid chromatography coupled with quadrupole mass spectrometry (UPLC-MS) was applied to determinate the concentration of BAs, which was followed by multivariate statistical analysis of Principal Component Analysis (PCA) and Partial Least Squares Discriminate Analysis (PLS-DA).

Results: Based on PCA results, three groups (Vehicle group, ANIT group and RhO + ANIT group) were clearly distinguished. Tauro-cholic acid (TCA), tauro-hyodesoxycholic acid (THDCA), glyco-cholic acid (GCA), and glyco-chenodeoxycholic acid (GCDCA) were proved to be the most important markers corresponding to ANIT-induced liver injury and protection provided by Rhubarb, which is further confirmed by PLS-DA. A correlation was found between the foregoing BAs and biochemical parameters including serum aspartate aminotransferase (ALT) and aspartate aminotransferase (AST), which confirmed that TCA, THDAC, GCA, and GCDCA could be considered as sensitive biomarkers.

Conclusion: The variance of the BAs contents can be used to evaluate ANIT-induced hepatotoxicity caused by ANIT and protective effects of Rhubarb. It also lays the foundation for the further research on the mechanisms of cholestasis as well as the therapeutic effect of Rhubarb.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jep.2012.09.049>.

Keywords

Bile acids; Rhei radix *et* Rhizoma; Rhubarb; α -naphthylisothiocyanate; Hepatoprotective effect; Multivariate statistical analysis

1. Introduction

Cholestasis can be defined as a condition with a defective bile acid (BAs) flow from the liver to the duodenum. There are two obstructive types of cholestasis. One is a mechanical blockage in the duct system, and the other is a metabolic disorder induced by genetic defects or side effects of many medications that disturb the bile generation (Boyer, 2007). There is no effective therapeutic drug in clinical application, and only Urso (ursodeoxycholic acid, UDCA) is available to reduce cholesterol absorption. Therefore, it is crucial to identify appropriate therapeutic agents for cholestatic liver diseases (Ding et al., 2006).

The rodents treated with α -naphthylisothiocyanate (ANIT) are commonly used as animal models for cholestasis research. ANIT is a compound known to cause acute hepatobiliary toxicity in vivo characterized by infiltration of neutrophils around bile ducts and severe interlobular duct epithelial necrosis (Plaa and Priatly, 1977). Meanwhile, ANIT is metabolized by cytochrome P450 and its metabolites are conjugated with glutathione. The conjugated ANIT is transported into the bile to damage the bile duct epithelial cells and thus lead to cholestatic hepatitis.

Traditional Chinese Medicine (TCM) has a long history as effective agents for treating liver disorders, including cholestasis. Rhubarb (Rhei Radix *et* Rhizoma), the underground parts of *Rheum palmatum* L., *Rheum tanguticum* Maxim. *ex* Balf., or *Rheum officinale* Baill. (China Pharmacopoeia Committee, 2010) has been used for hundreds of years in China to remove accumulation with purgation, clear heat and purge fire, cool the blood and remove toxin, expel stasis to unblock the meridian, and drain dampness to abate jaundice (China Pharmacopoeia Committee, 2010). Pharmacological studies showed that this herb indicated broad spectral activities, such as purgative and cathartic (Xiao et al., 1984), anti-inflammatory (Li et al., 2005; Chen et al., 2004), anti-oxidative, and anti-microbial effects (Zhao et al., 2009; Jung et al., 2004), and was applied for liver protection, treatment of cholestatic hepatitis and gastrointestinal diseases in clinical practice, but with unclear mechanism (Arosio et al., 2000).

BAs are the main endogenous ingredients in bile, which are synthesized and secreted by hepatocytes into the bile canaliculus. The primary BAs, including cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized from cholesterol and conjugated with glycine and taurine in the liver. After secreted into the intestine, 25% of the primary BAs are de-conjugated and dyhydroxylated by intestinal bacterial enzymes and formed the secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA). Both primary and secondary BAs are subject to enterohepatic circulation. However, when the insults of xenobiotics incur liver damage, the variances in BAs are related to the physiological status of the liver. Therefore, to some extent, the metabolic profiling of BAs can reflect the liver function and contribute significantly to the mechanism of cholestasis. In our recent research, an ultra-

performance liquid chromatography–mass spectrometry (UPLC–MS) method has already been established and validated for simultaneous analysis of 22 BAs and is applied to the toxicity evaluation on carbon tetrachloride (CCl₄)- and ANIT-induced liver failure in rats (Yang et al., 2008).

In the present study, the protective effect of Rhubarb against ANIT-induced cholestasis in rats was evaluated based on the metabolic profile of BAs using UPLC-MS, to afford the understanding of the mechanism-based hepatoprotective and choleric effect of Rhubarb.

2. Materials and methods

2.1. Materials and instruments

Rhubarb was provided by Shanghai Hutchlson Pharmaceuticals Company and authenticated as *R. officinale* Baill. by Dr. Lihong Wu by referring the description in the monograph of *Rhei Radix et Rhizoma* (China Pharmacopoeia Committee, 2010), and by direct comparison of the macro- and micro-characteristics with an authentic sample. The voucher specimens (dh-110305) were deposited in the Herbarium of Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine. CMC-Na was purchased from Sinopharm Chemical Reagent Co., Ltd. Cholic acid (CA), deoxycholic acid (DCA), chenocholic acid (CDCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), hyodesoxycholic acid (HDCA), glycocholic acid (GCA), taurocholic acid (TCA), glycodeoxycholic (GDCA), taurodeoxycholic acid (TDCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), glyoursodeoxycholic acid (GUDCA), taoursodeoxycholic acid (TUDCA), taurohyodesoxycholic acid (THDCA), glycolithocholic acid (GLCA), tauroolithocholic acid (TLCA) and ANIT were purchased from Sigma-Aldrich. Ammonium acetate, formic acid and acetonitrile (HPLC-grade) were purchased from Fisher Scientific (Nepean, Ont., Canada). HPLC-grade methanol was purchased from Tedia Inc. Ultrapure water was purified with a Milli-Q system (Millipore, Bedford, MA). The other solvents of analytical grade were obtained from Shanghai Chemical Factory (Shanghai, China).

2.2. Animals

Male Wistar rats (clean grade, 200–240 g, 6–8 weeks of age) were obtained from the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine (SHUTCM, Shanghai). The conditions were maintained thermostatically at $(20 \pm 2)^\circ\text{C}$ with relative humidity at 60%–70%. Animal welfare and experimental protocols were strictly complied with the Guide for the Care and Use of Laboratory Animals and the ethics and regulations of SHUTCM.

2.3. Animals treatment and sample collection

The rats were randomly assigned into three groups, normal control group (Vehicle $n = 8$), ANIT treated ($n = 12$), and Rhubarb treated groups (RhO + ANIT, $n = 12$). The Vehicle and the ANIT groups were intragastrically administered with normal saline (NS) in first 7 days, and meantime the rats in RhO + ANIT group were intragastrically administered with Rhubarb powder dissolved in aqueous solution (5 g/kg, 13.3 ml/kg). In the 8th day, the

ANIT and the RhO + ANIT groups were intragastrically administered with ANIT (dissolved in oil, 65 mg/kg), while the Vehicle group was given soybean oil (65 mg/kg) intragastrically. After administration, the rats were provided with food and water normally and were fasted for twelve hours before the sacrifice. The rats were sacrificed at 48 h after the administration of ANIT (in the 10th day) and their livers and blood were collected. The blood samples were coagulated in 1.5 h and centrifuged to obtain serum at 4 °C for 10 min ($3000 \times g$). Each liver was isolated and stored at $-80\text{ }^{\circ}\text{C}$ for further analysis, except for the left lobe, which was used for histological studies.

2.4. Biochemical assay and histological study

The serum was used to test the levels of serum aspartate aminotransferase (AST), albumin (ALB), total bilirubin (TBIL), aspartate aminotransferase (ALT), and triglyceride (TG). Sections of rat liver stained with a standard hematoxylin and eosin (HE) procedure.

2.5. Quantitative determination

The quantification method (Yang et al., 2008) was conducted with modification.

Preparation of standard solutions: The mix reference standards were prepared by dissolving each bile acid reference standard respectively in methanol. The calibration standards were prepared from 0.01 to $10\text{ }\mu\text{g mL}^{-1}$.

2.5.1. Sample preparation: The UPLC–MS method was modified to determine the contents of BAs (Yang et al., 2008). A $200\text{ }\mu\text{L}$ of serum was de-proteinized by adding $600\text{ }\mu\text{L}$ of methanol. The mixture was agitated for 1 min using a vortex agitator. After centrifugation at $20,000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$, $650\text{ }\mu\text{L}$ of the supernatant was collected and blown to dryness under nitrogen. The residue was dissolved in a $100\text{ }\mu\text{L}$ mixture of 0.01% acetic acid containing 5 mM ammonium acetate/methanol (45:55, v/v) and centrifuged at $20,000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$. A $5\text{ }\mu\text{L}$ of the supernatant was applied to the UPLC–MS system for analysis.

2.5.2. Chromatographic condition: The BAs were quantified by a Waters Acquity UPLC System coupled with a ZQ 2000 quadrupole spectrometry (Waters, MA, US). An ACQUITY UPLC BEH column ($1.7\text{ }\mu\text{m}$, $2.1\text{ mm} \times 100\text{ mm}$) heated to $45\text{ }^{\circ}\text{C}$ was used for chromatographic separation. A gradient system consisted of the Solvent A (methanol) and the Solvent B (0.01% acetic acid containing 5 mM ammonium acetate) at a flow rate of 0.3 mL/min , the gradient program used was as following: 55% A (0–1.0 min), 55–62% A (1.0–2.6 min), and 62–80% A (2.6–11.4 min). The injection volume was $5\text{ }\mu\text{L}$. The mass spectrometer was used in Selective Ion Monitoring (SIM) function in the ESI-negative mode. The capillary voltage was set at 3.0 kV, and the cone voltage was 55 V. The source temperature was $120\text{ }^{\circ}\text{C}$, and the desolvation temperature was $300\text{ }^{\circ}\text{C}$, while the desolvation gas flow was set to 700 L/h , and the cone gas flow was 50 L/h .

2.6. Statistical analysis

UPLC–MS data were acquired and processed using a MassLynx 4.1 software. SPSS 11.5 software (SPSS, Inc., Chicago, US) and Simca-P 12.0 (Umetrics, Umea, Sweden) were used to run TURKEY's test, PCA and PLS-DA analyses.

3. Results

3.1. Assay of biochemical indicators

Serum ALT and AST activities, two common clinical biomarkers of liver injury, along with other biochemical indicators were examined, as shown in Fig. 1 and Supplement Table 1.

At 48 h after the treatment of ANIT, the serum TBIL level of the ANIT group was elevated by 300-fold compared with that of the Vehicle group and the activities of ALT and AST were also significantly increased, which indicated that severe cholestasis occurred. However, the levels of the RhO + ANIT group declined significantly compared with those of the ANIT group. The overall biochemical data suggested that the animal model of cholestatic liver injury induced by ANIT was successfully established and Rhubarb showed obvious protective effect against the injury.

3.2. Histopathological examination

The liver sections stained with HE were examined for the histopathological assessment of ANIT-induced liver injury and the protective effects of Rhubarb (Fig. 2). Marked cholestasis in intrahepatic bile ducts and hepatocytes necrosis as well as dilatation of blood vessels and hyperplasia of biliary epithelium were clearly distinguishable in ANIT-challenged rats (Fig. 2B), compared with the normal liver morphologies in the Vehicle group (Fig. 2A). Samples from the RhO + ANIT group exhibited reduced liver damage (Fig. 2C). Cholestasis in intrahepatic bile duct was mitigated and the hepatocyte necrosis or the biliary epithelium hyperplasia was not obvious. It was suggested that Rhubarb ameliorated the cholestatic liver injury.

3.3. Bile acids contents

A validated UPLC–MS method was applied to determine the contents of individual BAs in rat serum. A total of seventeen BAs were simultaneously quantified in the serum of the Vehicle, ANIT and RhO + ANIT treated groups and the contents of BAs were summarized (Fig. 3 and Supplement Table. 2).

The results showed that BAs concentrations varied significantly in the three groups. The concentration of the most conjugated BAs increased, while the free BAs decreased in the ANIT group. For example, taurochenodeoxycholic acid (TCDCA) was increased by 101-fold and taurocholic acid (TCA) by 198-fold, whereas deoxycholic acid (DCA) was decreased by 16-fold and ursodeoxycholic acid (UDCA) by 26-fold, compared with the Vehicle group, respectively (Supplement Table. 2). Comparing with the ANIT group, the reversal tendency of tauroursodeoxycholic acid (TUDCA), taurohyodesoxycholic acid (THDCA), TCA, glycocholic acid (GCA), TCDCA, taurodeoxycholic acid (TDCA),

ursodeoxycholic acid (UDCA), glycochenodeoxycholic acid (GCDCA) was observed in the RhO + ANIT group.

3.4. PCA and PLS-DA analysis

The datasets of the Vehicle, ANIT, and RhO + ANIT treated groups were analyzed by PCA and PLS-DA. The parameters used to assess modeling quality included R^2X , R^2Y , and Q^2Y . The ANIT model parameters for the explained variation, R^2 , and the predictive capability, Q^2 , were high (R^2 , $Q^2 > 0.5$), indicating the model was successfully established. Clear separation was observed among the three groups in the score plot with 17 BAs variables. The ANIT group was clustered together and relatively far from that of the Vehicle group, while the Vehicle group was scattered a little (Fig. 4). It also indicated the trend of recovery when using Rhubarb against ANIT-induced liver injury in rats (Fig. 4). The RhO + ANIT group were aggregated in a narrow region between those of the ANIT group and the Vehicle group. The results suggested that the predictability of 17 types of BAs to explain the model of the three groups was acceptable, with the values of R^2X , R^2Y , and Q^2Y as 75%, 62.4%, and 57.7%, respectively. Furthermore, to identify the most potential biomarkers of variables, the VIP > 1 values were selected. Then CA, TCA, DCA, CDCA, THDCA, HDCA, GCA and GCDCA were screened out as the candidates. With the eight variables above, the analysis was applied with PLS-DA again. The predictability of this model was improved significantly according to the parameters of R^2X , R^2Y , and Q^2Y (83.9%, 64.7% and 61.7%, respectively). Furthermore, in combination with the results of BAs in serum and the significant statistical differences ($P > 0.05$) TCA, THDCA, GCA, and GCDCA, among the 17 types of BAs, served as potential biomarker to discriminate the three groups as well as to evaluate the protection of Rhubarb in the ANIT-induced model.

3.5. Correlation analysis

To further clarify the potential biomarkers to evaluate the effect of Rhubarb, we performed the correlation analysis which was verified between bile acids group (CA, TCA, DCA, CDCA, THDCA, HDCA, GCA, GCDCA) and biochemical indicators (ALB, ALT, AST, TBIL, TG). As shown in the Table 1, the profile of eight types of BAs was closely correlated with the levels of ALT, AST and TBIL, whereas the BAs did not reveal correlations with the levels of TG, but were correlated with that of ALB except CA, DCA and HDCA.

3.6. Hierarchical cluster method

In hierarchical cluster analysis, scaled expression values were color-coded. While the red end implicates higher values, and the green end implicates the opposite. Therefore, the different ratios of BAs in three groups were distinguished through their color gradients. Based on the relationship of different BAs, it is of certain significance to utilize CA/CDCA, CA/UDCA, CA/DCA, TUDCA/UDCA, TDCA/DCA, and GDCA/DCA to discriminate the difference in the three groups, and further to evaluate the protection of Rhubarb. Accordingly, as shown in Fig. 5, significant changes were revealed by the ratios of BAs with the liver injury induced by ANIT and the liver protection by Rhubarb.

4. Discussion

The liver is the target organ which is responsible for the metabolism of drugs or toxic substances including ANIT (Bissel et al., 2001). Although extensive research has been performed including the mechanisms of liver injuries induced by drugs or chemicals, the lack of effective therapeutic medicines or programs to treat the liver injuries still exists. Rhubarb has long been used for the treatment of cholestatic hepatitis and gastrointestinal diseases. The principal bioactive components of Rhubarb has been reported to be the anthraquinones including aloe-emodin, rhein, emodin, chrysophenol, physcion, and their glycosides such as 8-*O*- β -D-glucoside, as well as sennosides A and B. As recorded in China Pharmacopoeia Committee, 2010, Rhubarb (*Rhei Radix et Rhizoma*) is derived from three *Rheum* species: *R. palmatum* L., *R. tanguticum* Maxim. ex Balf., or *R. officinale* Baill., yet the *R. officinale* Baill., are more popularly used due to its relative abundant resources. Therefore in this study, the underground parts of *R. officinale* Baill. were selected as the experimental materials for the protection against the ANIT-induced liver injury at a dose of 5 g/kg by referring to the reported literatures (Yan et al., 2009, Xing et al., 2011).

In this study, histopathological examination demonstrated that the marked cholestasis in intrahepatic bile ducts was clearly distinguishable in the ANIT model group. In addition, the extent of cholestasis was alleviated in the RhO + ANIT group. The serum levels of biochemical indicators including TBIL, ALT and AST also suggested that ANIT-induced hepatocytes damage and cholestasis, and whereas Rhubarb showed protective effects, which was consistent with the results of the histopathological analysis.

Moreover, metabolic profiling of BAs was applied to discriminate the three groups. BAs maintain equilibrium in the peripheral circulation with mutual transformation between the free and conjugated forms. When the hepatic or intestinal diseases occur, it can break the BAs equilibrium, especially on the synthesis, clearance and absorption of BAs, which can lead to the elevated levels of total BAs. The contents of TCA, GCA, and GCDCA are distinctly higher in intrahepatic cholestasis of pregnancy than those of normal one (Meng et al., 1997). Furthermore, it has been reported that the accumulation of DCA, CDCA, LCA, CA, and their conjugates can lead to hepatic damage (Palmeira and Rolo, 2004). Similarly, the present study showed that taurine conjugates (TCA, THDCA) and glycine conjugates (GCA, GCDCA) were identified as the important biomarker to discriminate the three groups as well as to evaluate the protection of Rhubarb in the ANIT-induced model.

After the C₂₄ site taurine-combination, an analysis from the molecular structure of BAs reveals that these conjugates have a negative charge with highly ionization that causes the impermeability of cell membrane. At the same time the conjugates have a large volume and are hard to get through the membrane of bile ducts, so the fact that intrahepatic cholestasis induced by BAs conjugates can be attributed to the impermeability of cell membrane (Hofmann, 1999). In the normal circumstance, the contents of BAs in serum are low and stable, but when the pathological condition occurs, the concentration of BAs fluctuates distinctly. After administration of ANIT, the bile ducts are obstructed and the injuries occur with biochemical function alteration of enzymes in bile duct membrane, and further inhibit the excretion of taurine conjugates. The molecules of glycine conjugates are so small that

the inhibitory extent in transportation is less serious than that of the taurine conjugates. Previous reports suggested that BAs are excreted into the liver tissue mainly through Na⁺-taurocholate co-transporting polypeptide (ntcp) and organic anion transporting polypeptide 1 (oatp1) (Zimmerli et al., 1989; Kouzuki et al., 2000), which ntcp acts on dominant action for taurine-conjugated BAs (Schroeder et al., 1998). Therefore, the taurine conjugates are more sensitive to reflect the changes in ambient environment than the glycine conjugates accompanying, which can be proved in this study. Other factors, such as multidrug resistance proteins (mrp3 and/or mrp2), which transports BAs out of cells (Bodo and Bakos, 2003) and prevent intracellular BAs overload, have a high affinity for TCA and GCA. So it can explain the significant increased TCA and GCA in ANIT group. Furthermore, Rhubarb has a restoring activity on cholestatic hepatitis based on the contents of GCA, GCDCA, THDCA, and TCA (Fig. 3) and the mechanism of the protection by Rhubarb can be associated with the transporting BAs through the related transporter protein.

In the present study, it was suggested that GCA, GCDCA, THDCA, and TCA were the sensitive and potential biomarkers in the protection of Rhubarb against ANIT-induced cholestasis. Apart from that, it was the first time that we used of the ratios to characterize the cholestasis and evaluate the effect of Rhubarb including CA/CDCA, CA/UDCA, CA/DCA, TUDCA/UDCA, TDCA/DCA, GDCA/DCA, which offered further evidence to diagnose the liver injury. The responsive varieties of BAs provided a reason to regard the BAs as the biochemical indicators. Furthermore, the results obtained in the current study suggested that Rhubarb contributed the most to regulating the transportation of the BAs, and it can provide the potential interpretation of hepatoprotective effects of Rhubarb.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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References

- Arosio B, Gagliano N, Fusaro LM, et al., 2000 Aloe Emodin quinone pretreatment reduces acute liver injury induced by carbon tetrachloride. *Journal of Pharmacological and Toxicological Methods* 87, 229–233.
- Bissel DM, Gores GJ, Laskin DL, Hoorhagel JH, 2001 Drug induced liver injury: mechanisms and test systems. *Hepatology* 33, 1009–1013. [PubMed: 11283870]
- Bodo A, Bakos E, 2003 Differential modulation of the human liver conjugate transporters MRP2 and MRP3 by bile acids and organic anions. *Journal of Biological Chemistry* 235, 29–37.
- Boyer JL, 2007 New perspectives for the treatment of cholestasis: lessons from basic science applied clinically. *Journal of Hepatology* 46, 365–371. [PubMed: 17223219]
- Chen RF, Shen YC, Huang HS, Liao JF, Ho LK, Chou YC, Wang WY, Chen CF, 2004 Evaluation of the anti-inflammatory and cytotoxic effects of anthraquinones and anthracenes derivatives in human leucocytes. *Journal of Pharmacy and Pharmacology* 56, 915–919. [PubMed: 15233871]

- China Pharmacopoeia Committee, 2010 Chinese Pharmacopoeia, vol. 1 China Chemical Industry Press, Beijing, pp. 22–23.
- Ding Y, Zhao L, Mei H, Huang ZH, Zhang SL, 2006 Alterations of biliary biochemical constituents and cytokines in infantile hepatitis syndrome. *World Journal of Gastroenterology* 12, 7038–7041. [PubMed: 17109502]
- Hofmann AF, 1999 The continuing importance of bile acids in liver and intestinal disease. *Archives of Internal Medicine* 159, 2647–2658. [PubMed: 10597755]
- Jung HA, Chung HY, Yokozawa T, Kim YC, Hyun SK, Choi JS, 2004 Alaternin and emodin with hydroxyl radical inhibitory and/or scavenging activities and hepatoprotective activity on tacrine-induced cytotoxicity in HepG2 cells. *Archives of Pharmacal Research* 27, 947–953. [PubMed: 15473666]
- Kouzuki H, Suzuki H, Sugiyama Y, 2000 Pharmacokinetic study of the hepatobiliary transport of indomethacin. *Pharmaceutical Research* 17, 432–438. [PubMed: 10870987]
- Li HL, Chen HL, Li H, Zhang KL, Chen XY, Wang XW, Kong QY, Liu J, 2005 Regulatory effects of emodin on NF-kappaB activation and inflammatory cytokine expression in RAW 264.7 macrophages. *International Journal of Molecular Medicine* 16, 41–47. [PubMed: 15942676]
- Meng LJ, Reyes H, Palma J, Hernandez I, Ribalta J, Sjovall J, 1997 Profiles of bile acids and progesterone metabolites in the urine and serum of women with intrahepatic cholestasis of pregnancy. *Journal of Hepatology* 27, 346–357. [PubMed: 9288610]
- Palmeira CM, Rolo AP, 2004 Mitochondrially-mediated toxicity of bile acids. *Toxicology* 203, 1–15. [PubMed: 15363577]
- Plaa GL, Priatly BG, 1977 Intrahepatic cholestasis induced by drugs and chemicals. *Pharmaceutical Research* 28, 207–273.
- Schroeder A, Eckhardt V, Stieger B, Tynes R, Schteingart CD, Hofmann AF, Meier PJ, Hagenbuch B, 1998 Substrate specificity of the rat liver Na(+)- bile salt cotransporter in *Xenopus laevis* oocytes and in CHO cells. *American Journal of Physiology* 274, 370–375.
- Xiao P, He L, Wang L, 1984 Ethnopharmacologic study of Chinese rhubarb. *Journal of Ethnopharmacology* 10, 275–293. [PubMed: 6748707]
- Xing XY, Zhao YL, Kong WJ, Wang JB, Lei J, Ping Z, Dan Y, Zhong YW, Li RS, Xiao XH, 2011 Investigation of the “dose–time–response” relationships of rhubarb on carbon tetrachloride-induced liver injury in rats. *Journal of Ethnopharmacology* 135, 575–581. [PubMed: 21463673]
- Yan LZ, Jia BW, Guang DZ, Li MS, Xiao HX, 2009 Investigations of Free anthraquinones from Rhubarb against -naphthylisothiocyanate-induced cholestatic liver injury in rats. *Basic and Clinical Pharmacology and Toxicology* 104, 463–469. [PubMed: 19389047]
- Yang L, Xiong AZ, He YQ, 2008 Bile acids metabonomic study on the CCl4- and alpha-naphthylisothiocyanate-induced animal models: quantitative analysis of 22 bile acids by ultraperformance liquid chromatography-mass spectrometry. *Chemical Research in Toxicology* 21, 2280–2288. [PubMed: 19053324]
- Zimmerli B, Valantinas J, Meier PJ, 1989 Multispecificity of Na +-dependent taurocholate uptake in basolateral (sinusoidal) rat liver plasma membrane vesicles. *Journal of Pharmacology and Experimental Therapeutics* 250, 301–308. [PubMed: 2746502]
- Zhao YL, Wang JB, Zhou GD, Shan LM, Xiao XH, 2009 Investigations of free anthraquinones from rhubarb against naphthylisothiocyanate-induced cholestatic liver injury in rats. *Basic and Clinical Pharmacology and Toxicology* 104, 463–469. [PubMed: 19389047]

Fig. 1. Serum levels of AST, ALT, ALB and TBIL. Significant difference between two groups, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 2. Liver tissue sections from normal rats (A), rats receiving ANIT (B) and rats treated with Rhubarb (C). (hematoxylin and eosin stained, original magnification $\times 200$).

Fig. 3.

The contents of UDCA, GCA, GCDCA, TDCA, TUDCA, TCDCA, THDCA, TCA in rats serum, significant difference between two groups, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 4. Results of PLS-DA analysis between vehicle rats groups (triangle marked), ANIT rats groups (dot marked) and RhO + ANIT rats groups (diamond marked) $t[1]$ and $t[2]$ are the first two eigenvectors of original data.

Fig. 5. Heatmap of the BAs determined by UPLC-MS in the serum. The heatmap graphically represents the ratio of BAs changes of abundance. The rows represent the groups of different treatments. The columns represent the ratio of BAs observation.

Table 1

Results of correlation analysis containing correlation coefficient.

BA	ANIT(ALT)		ANIT(AST)		ANIT(ALB)		ANIT(TBIL)		ANIT(TG)	
	r	p	r	p	R	p	r	P	r	p
CA	0.610	0.000 ^a	0.754	0.000 ^a	0.036	0.852	0.333	0.072	-0.219	0.244
TCA	0.817	0.000 ^a	0.801	0.000 ^a	-0.590	0.001 ^b	0.811	0.000 ^a	-0.179	0.354
DCA	0.525	0.003 ^b	0.612	0.000 ^a	-0.023	0.902	0.441	0.015	-0.386	0.035 ^c
CDCA	-0.475	0.008 ^b	-0.497	0.005 ^b	0.579	0.001 ^b	-0.717	0.000 ^a	-0.100	0.601
THDCA	-0.212	0.261	-0.222	0.238	0.551	0.002 ^b	-0.461	0.010 ^c	-0.300	0.107
HDCA	0.466	0.010 ^c	0.508	0.004 ^b	-0.043	0.821	0.428	0.018 ^c	-0.279	0.135
GCA	-0.540	0.002 ^b	-0.577	0.001 ^b	0.603	0.000 ^a	-0.718	0.000 ^a	-0.062	0.748
GCDC A	-0.531	0.003 ^b	-0.562 ^b	0.001 ^b	0.574	0.001 ^b	-0.716	0.000 ^a	-0.056	0.767

r: Pearson correlation coefficient.

^a $P < 0.001$ vs vehicle.^b $P < 0.01$.^c $P < 0.05$.